CHAPTER 6

Analysis and Biological Activities of Potato Glycoalkaloids, Calystegine Alkaloids, Phenolic Compounds, and Anthocyanins

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6.1 Introduction

This limited overview on the analysis of four classes of the following secondary potato metabolites is, except for anthocyanins, largely limited to our own studies of glycoalkaloids, calystegine alkaloids, and phenolic compounds. Because interest in these potato constituents arises from potential health benefits and occasional toxicity, we also include in this overview a brief discussion of these aspects that relate to composition and a description of experimental methods. The interested reader should consult the cited references for an entry into the extensive worldwide literature on the diverse analytical and biological aspects for these metabolites.

6.2 Glycoalkaloids

Steroidal glycoalkaloids are naturally occurring, secondary plant metabolites that are found in a number of foods including potatoes, tomatoes, and eggplants (reviewed in Friedman, 2002; Friedman and McDonald, 1997, 1999a, b). Although in high doses they are toxic, glycoalkaloids may also have beneficial effects. These include lowering of blood cholesterol (Friedman et al., 2000a, b), protection against infection by *Salmonella typhimurium* (Gubarev et al., 1998), and chemoprevention of cancer (Cham, 1994; Lee et al., 2004; Friedman et al., 2005, 2007).

In commercial potatoes (*Solanum tuberosum*) there are two major glycoalkaloids, α-chaconine and α-solanine, both trisaccharides of the common aglycone solanidine. These two compounds comprise about 95% of the glycoalkaloids in potato tubers. Their hydrolysis products, the β and γ forms and solanidine, may also be present in relatively insignificant concentrations. The structures of these glycoalkaloids and their hydrolysis products are presented in Figure 6.1.

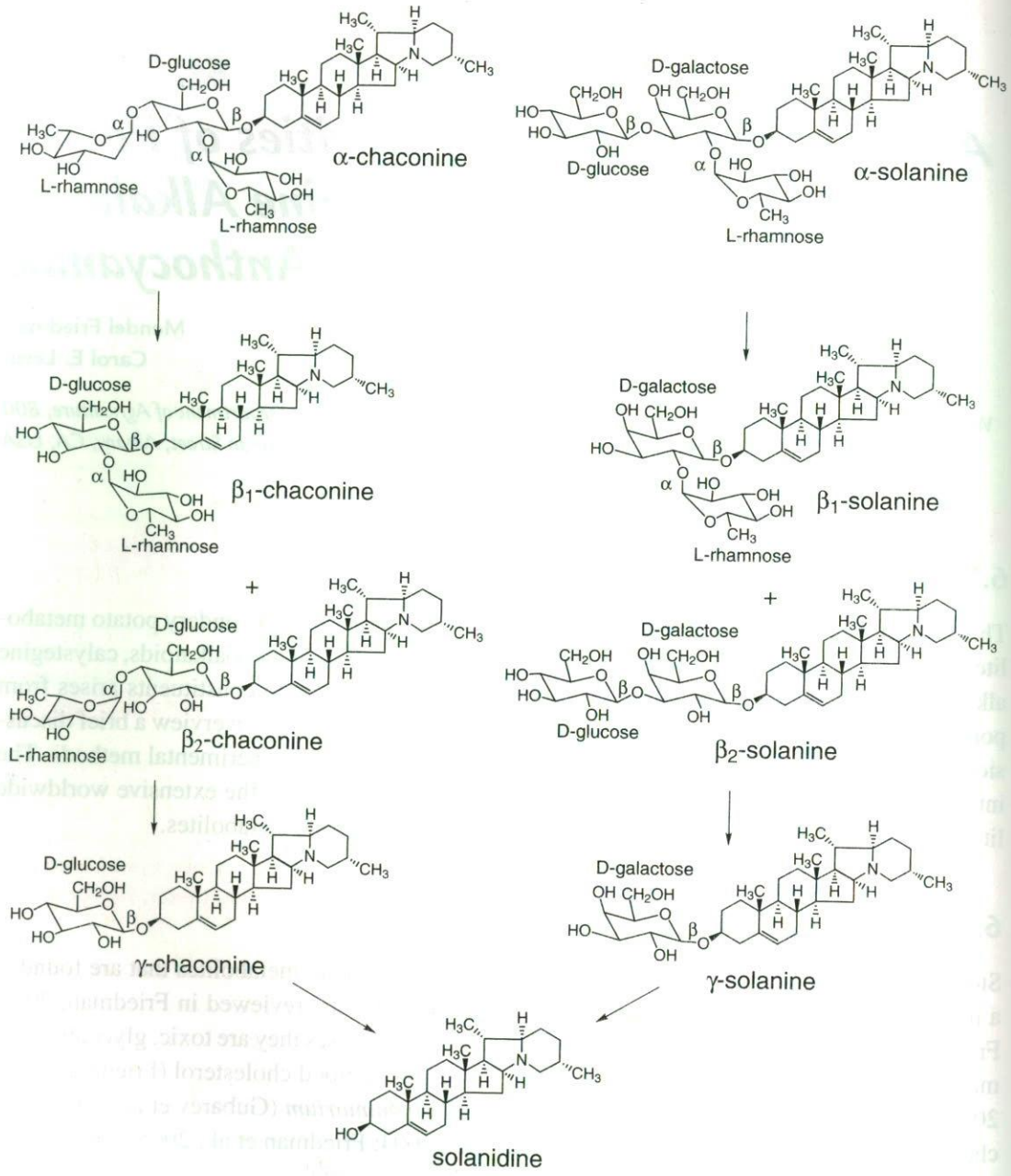


Figure 6.1: Structures of potato glycoalkaloids α-chaconine and α-solanine, and hydrolysis products (metabolites).

6.2.1 Toxicity

The toxicity of glycoalkaloids at appropriately high levels may be due to such adverse effects as anticholinesterase activity on the central nervous system (Abbott et al., 1960; Heftmann, 1967), induction of liver damage (Caldwell et al., 1991), and disruption of cell membranes adversely affecting the digestive system and general body metabolism (Keukens et al., 1995). At lower doses, the toxicity of glycoalkaloids in humans causes mainly gastrointestinal disturbances such as vomiting, diarrhea, and abdominal pain. At higher doses, it produces systemic toxicity, including symptoms such as fever, rapid pulse, low blood pressure, rapid respiration, and neurological disorders. Several cases of lethal poisoning have been reported (Korpan et al., 2004). There is some concern that dietary glycoalkaloids, due to their anticholinesterase properties, may adversely influence the actions of anesthetic drugs that are metabolized by acetylcholinesterase and butyrylcholinesterase (Krasowski et al., 1997). We found that solanidine exhibited estrogenic activity in an in vitro assay (Friedman et al., 2003a), with unknown health effects. Other complicating factors regarding the glycoalkaloid content of the diet that must be taken into account are: (a) α-chaconine appears to be more biologically active by a factor of about three to ten than is α-solanine; and (b) certain combinations of the two glycoalkaloids can act synergistically (Rayburn et al., 1995b; Smith et al., 2001).

These considerations have led to the establishment of informal guidelines limiting the total glycoalkaloid concentration of new potato cultivars to 200 mg/kg of fresh weight. As discussed elsewhere, these guidelines may be too high (Friedman, 2006; Friedman and McDonald, 1997). In one short-term clinical trial with human volunteers, one test subject experienced gastrointestinal disturbances after consuming mashed potatoes containing glycoalkaloids at the recommended limit, 200 mg/kg (Mensinga et al., 2005).

There have been few human studies, most of them anecdotal, so the susceptibility per individual variation and influence of other factors is not well established. The incidence of glycoalkaloid poisoning may be underreported, probably because physicians are more likely to implicate foodborne pathogens or general viral infections as the causative agents of gastrointestinal illness. Even these more common illnesses are known to be underreported. We therefore have no real basis to determine the frequency of poisoning caused by glycoalkaloids (Hopkins, 1995).

Toxicity may be influenced by other factors such as diet and general health. Glycoalkaloids are not well absorbed. However, the damaged intestinal wall may allow a spillover effect, causing them to be absorbed at a much faster rate after the mucosal cells have become compromised. This would account for the differences in symptoms observed for low (gastrointestinal) and for high (acute systemic) toxicity. It is worth noting that the apparent non-toxicity of the tomato glycoalkaloid α-tomatine appears to be due to complex formation with cholesterol in the digestive tract (Friedman et al., 2000b, 2007). Potato tubers of somatic hybrids whose progenies were the cultivated potato *Solanum tuberosum* and the wild-type *Solanum acaule* contained four glycoalkaloids, including tomatine, derived from the fusion parents (Kozukue et al., 1999).

These considerations suggest the possibility of developing high-tomatine potatoes for safety and health-promoting qualities (Friedman et al., 2007; Kozukue et al., 2008).

Other dietary factors, such as fiber, may also affect absorption in the gut. Hydrolysis products, the β-, γ-, and aglycone forms, are less toxic than the α-form (Rayburn et al., 1994). Therefore, processes that induce hydrolysis of the α-forms, such as exposure to *Aspergillus niger* (Laha and Basu, 1983) or to acid pH, may decrease toxicity. Other components and nutrients in the blood stream my affect toxicity as well. Folic acid, glucose-6-phosphate, and nicotine adenine dinucleotide (NADP) are reported to protect frog embryos against α-chaconine-induced developmental toxicity (Rayburn et al., 1995a; Friedman et al., 1997). Folic acid is now widely consumed by pregnant women to protect the fetus from neural tube malformations. It is also worth noting that Renwick (1972) found an epidemiological correlation between the congenital neural malformations anencephaly and spina bifida in fetuses and consumption of blighted potatoes by their mothers.

6.2.2 Analysis

Glycoalkaloids appear to be largely unaffected by food processing conditions such as baking, cooking, and frying. The content of glycoalkaloids can vary greatly in different potato cultivars (Friedman and Dao, 1992). Additionally, growing conditions and post-harvest exposure to light, mechanical injury, and storage can enhance glycoalkaloid levels (Kozukue et al., 1993; Machado et al., 2007).

The complex nature of the glycoalkaloid–dietary relationship suggests the need for accurate methods to measure the content of individual glycoalkaloids and their metabolites in fresh and processed potatoes as well as in body fluids such as plasma and tissues such as liver. HPLC methods are now widely used to determine the concentrations of individual glycoalkaloids, as well as glycoalkaloid hydrolysis (glycolysis) products in fresh and processed potatoes and in different parts of the potato plant such as leaves and sprouts (Bushway, 1982; Bushway et al., 1986; Carman et al., 1986; Kozukue et al., 1987, 1999, 2001; Saito et al., 1990; Dao and Friedman, 1992, 1994, 1996; Friedman and Levin, 1992; Hellenäs et al., 1992; Friedman et al., 1993; Houben and Brunt, 1994; Friedman and McDonald, 1995; Kubo and Fukuhara, 1996; Panovska et al., 1997; Friedman et al., 1998; Brown et al., 1999; Kuronen et al., 1999; Nitithamyong et al., 1999; Bodart et al., 2000; Simonovska and Vovk, 2000; Sotelo and Serrano, 2000; Väänänen et al., 2000; Fragoyiannis et al., 2001; Esposito et al., 2002). Other possible methods include ELISA and the use of biosensors. We will now describe selected methods for analysis of glycoalkaloids.

6.2.2.1 HPLC analysis

The glycoalkaloids and their hydrolysis products, with the exception of the aglycone, are analyzable in a single isocratic run. Most analyses were done on reverse phase columns. Separation

on reverse phases is based on differences in polarity of the molecules provided by the sugar(s) attached to the aglycone. The most polar α-glycoalkaloids elute first, followed by the β- and γ-glycoalkaloids. The aglycone elutes very late, if at all under isocratic conditions. Analysis of all compounds can be achieved in a single HPLC run using gradient elution.

Previously, we found that silanol moieties remaining on silica packing after bonding the C18 or C8 functional groups, and after endcapping, strongly influenced retention and separation of potato glycoalkaloids (Friedman and Levin, 1992). Because the secondary interactions of the packing with these compounds improved the separation allowing resolution of α-solanine, α-chaconine, and hydrolysis products to baseline, we selected columns with less endcapping and more acidic characteristics. Recent advances in column packing technology have led to multifunctional columns that take advantage of these mixed mode separations, finely controlling the mix between hydrophobic, hydrophilic, and specialized column interactions. Better amino functional group (NH2) columns are now also available. In the following 'Methods' section, we describe two HPLC methods. The first method was developed in our laboratory using a highly acidic C18 column, and the second was developed by our colleague in Korea using an amino column. Both methods produce good separation and have different advantages. Although the amino column technique has longer run times, it is more adaptable to HPLC/MS.

6.2.2.2 ELISA analysis

A simple method that can analyze a large number of samples in a reasonably short time is needed to meet the needs of plant breeders, plant molecular biologists, food processors, and scientists interested in better defining the role of glycoalkaloids in the plant, in the diet, and in medicine. A reliable immunoassay that correlates with HPLC may provide the answer. An ELISA kit for glycoalkaloids that meets these criteria should find widespread use. To meet this need, we evaluated a prototype ELISA kit produced by EnviroLogix, Inc., Portland, Maine, based on antibodies we developed (Stanker et al., 1994, 1996, 1997). Our results show a good correlation between HPLC and the ELISA for glycoalkaloids in potato tubers and processed products (Table 6.1). ELISA is a simple method that can be used routinely by a broad range of users (Sporns et al., 1996; Friedman et al., 1998).

6.2.2.3 Biosensors

Researchers (Benilova et al., 2006; Arkhypova et al., 2008) are developing a biosensor-based pH-sensitive field-effect transistor technology for rapid determination of glycoalkaloids. The test takes advantage of the anticholinesterase activity of the glycoalkaloids. These tests could hold great promise, analogous to the ELISA test mentioned above.

6.2.3 Methods

The following is a description of the methods we used to analyze glycoalkaloids.

Table 6.1: Comparison of glycoalkaloid content of the same potatoes and potato products analyzed by HPLC (sum of α-chaconine and α-solanine) and ELISA

Sample	Assay method	
	HPLC	ELISA
Whole potatoes:	fresh (mg/kg)	Fresh (mg/kg)
Russet, organic	5.8	5.1
Russet	22	24
Yukon Gold	40	38
Purple, small	45	37
Red, small	101	128
Gold, small	105	113
White, large	125	132
White small	203	209
Potato plant parts:	dehydrated (mg/kg)	dehydrated (mg/kg)
Flesh, Red Lasoda	45.6	51.6
Peel, Shepody	1432	1251
Sprouts, Shepody	7641	6218
Leaves	9082	8851
Processed potatoes:	original (mg/kg)	original (mg/kg)
French fries, A	0	1.2
French fries, B	24.1	22.7
Chips, low-fat	15.2	22.7
Skins, A	43.3	35.0
Skins, B	37.2	41.0

6.2.3.1 *Extraction of glycoalkaloids from freeze-dried potatoes for HPLC*

Freeze-dried potato powders (17 mg–1 g, depending on availability) were extracted (Friedman et al., 2003c) with 40 mL of 5% acetic acid accompanied by ultrasonication for 10 min at room temperature. After filtration through a 3G3-glass filter, the residue was rinsed three times with 30 mL of 5% acetic acid each time. The washings were combined with the original filtrate. The filtrate was transferred to a 200-mL Erlenmeyer flask to which was added 10 mL of concentrated NH_4OH to precipitate the glycoalkaloids. The basic solution was placed in a 70°C water bath for 50 min and then refrigerated overnight. The precipitate was collected by centrifugation at 18 000 ***g*** for 10 min at 1°C and washed twice with a 2% solution of NH_4OH. The pellet was dried at 30°C under reduced pressure, then dissolved in 1 mL of a mixture of tetrahydrofuran/acetonitrile/20 mM KH_2PO_4, and centrifuged at 18 000 ***g*** for 10 min at 1°C. The supernatant (50 μL) was used for HPLC.

6.2.3.2 *HPLC technique, NH2 column*

Figure 6.2a shows the HPLC chromatogram using the NH2 column (Friedman et al., 2003c). HPLC chromatography was carried out with the aid of a Hitachi liquid chromatograph model

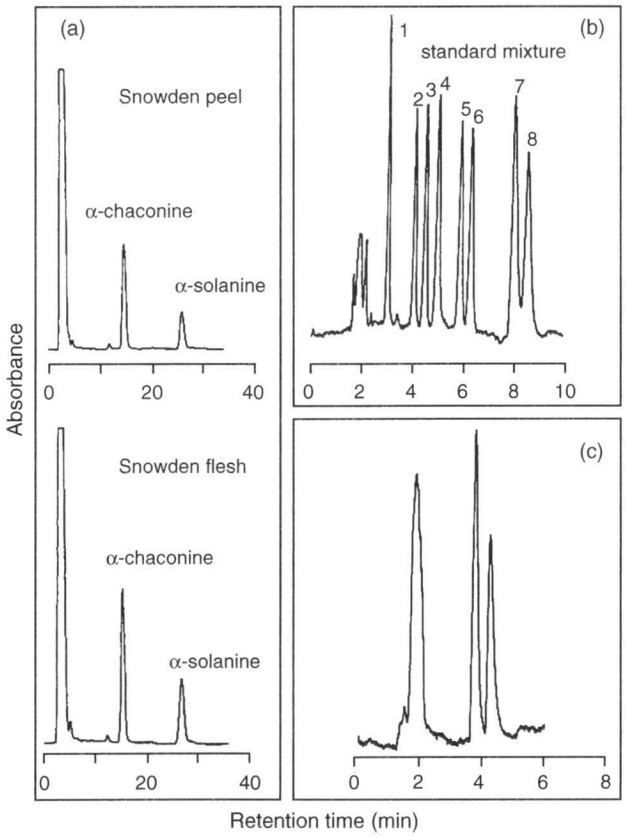


Figure 6.2: A comparison of HPLC separation methods. (a) HPLC of α-chaconine and α-solanine in the flesh and the peel of one variety of potato. Conditions: column, Inertsil NH2 (5 μm, 4.0 x 250 mm); mobile phase, acetonitile/20 mM KH_2PO_4 (80:20, v/v); flow rate, 1.0 mL/min; column temperature, 20°C; UV detector, 208 nm; sample size, 20 μL. (b) HPLC chromatogram of approximately 1 μg of each of potato glycoalkaloids and their hydrolysis products 1, solasonine (internal standard); 2, α-solanine; 3, α-chaconine; 4, β2-solanine; 5, β1-chaconine; 6, β2-chaconine; 7, γ-solanine; 8, γ-chaconine. Conditions: column, Resolve C18 (5 μm, 3.9 x 300 mm); mobile phase, 35% acetonitrile/100 mM ammonium phosphate (monobasic) at pH 3; flowrate, 1.0 mL/min; column temperature, ambient; UV detector, 200 nm; sample size. (c) HPLC chromatogram of the aglycones solanidine and solasodine. Conditions: column Supelcosil C18-DB (3 μm, 4.6 x 150 mm); mobile phase, 60% acetonitrile/10 mM ammonium phosphate pH 2.5; flowrate, 1.0 mL/min; column temperature, ambient; UV detector, 200 nm.

665A-11 equipped with a model 655-40 autosampler and a UV detector (Hitachi model 655A UV monitor) set at 208 nm. Column temperature was controlled with a Coolnics model CTR-120 device (Komatsu Electronics, Tokyo, Japan). Chromatogram peak areas were integrated with a Hitachi D-2500 chromato-integrator. The column was an Inertsil NH2 (5 μM, 4.0 × 250 mm)

(GL Science, Japan). The mobile phase was acetonitrile/20 mM KH_2PO_4 (80:20, v/v). For the aglycon solanidine, the mixture consisted of acetonitrile/2.5 mM KH_2PO_4 (93:7, v/v). The flow rate was 1 mL/min at a column temperature of 20°C. The concentrations of α-chaconine and α-solanine in the potato extracts were calculated by comparison with the integrated peak areas of known amounts of the standards by a Hitachi chromato-integrator.

6.2.3.3 HPLC technique, C18 column

Figures 6.2b and 6.2c show HPLC chromatograms using the C18 column. HPLC was carried out with the aid of a Beckman Model 334 liquid chromatograph with a 427 integrator and a 165 UV-visible variable wavelength detector set to 200 nm. The column was a Resolve C18 (5 μm, 3.9 × 300 mm) (Waters, Milford, MA). The mobile phase was 35% acetonitrile and 100 mM ammonium phosphate (monobasic) adjusted to pH 3.5 with phosphoric acid at a flow rate of 1 mL/min and ambient temperature. Concentrations of α-chaconine and α-solanine in the potato extracts were calculated by comparison of integrated peak areas with the peak of a solasonine using a Beckman 427 integrator. Conditions for solanidine were different due to the fact that it bound strongly to the C18 column and exhibited the significant peak tailing sometimes seen when basic compounds separated on acidic columns (Friedman and Levin, 1992). We used a Supelcosil Cl8 deactivated base, 3 μm, 4.6 × 150 mm column (Supelco Inc., Bellefonte, PA) with the following conditions: eluent, 60% acetonitrile/10 mM ammonium phosphate, pH 2.5.

6.2.3.4 Identification of glycoalkaloids

The two potato glycoalkaloids in the potato extract were identified as follows: (a) comparison of thin-layer chromatography of standards α-chaconine and α-solanine and of samples of each peak collected from the HPLC column containing the individual glycoalkaloids; and (b) HCl hydrolysis of the HPLC samples into sugars and the aglycon solanidine. These were identified by GLC-MS (Kozukue et al., 1999, 2008; Kozukue and Friedman, 2003).

6.2.4 Results

Table 6.2 contains a survey of glycoalkaloid content in potatoes analyzed using the above HPLC NH2 column technique. None of the whole potatoes exceeded the 200 mg total glycoalkaloids per kg of potatoes (see A + B column). However, this was not the case for potato peel. Five of the eight samples exceeded this benchmark. The high content of peels should not be of concern, unless consumers ate large amounts of peel, as they sometimes do in some commercial products, such as potato skin appetizers.

Researchers have proposed making use of that peel waste. Rodriguez de Sotillo proposed making use of the high antioxidant content of potato peel as an antimicrobial (Rodriguez de Sotillo et al., 1998). To prevent possible poisonings, care must be exercised when using the waste in human or animal food (Kling et al., 1986), or when releasing it into the environment as industrial waste.

Table 6.2: Glycoalkaloid and calystegine content of potato flesh, potato peel, and whole potatoes of eight potato cultivars (in mg/kg)

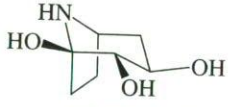
Potato cultivar		Glycoalkaloids				Calystegines			
		α-chaconine (A)	α-solanine (B)	A + B	A/B	calystegine A_3	calystegine B_2	$A_3 + B_2$	B_2/A_3
Atlantic	flesh	4.7	2.9	7.6	1.6	1.1	1.5	2.6	1.4
	Peel	8.8	3.6	12.4	2.4	31.2	141	172	4.5
	whole	5	8	8	1.7	3.5	12.9	16.4	3.7
Dark Red Norland	flesh	3.4	1.3	4.7	2.6	0	1.3	1.3	-
	Peel	128	60.3	188	2.1	6.4	33.3	39.7	5.2
	whole	16.8	7.7	24.5	2.2	0.7	4.7	5.4	6.7
Ranger Russet	flesh	12.8	7	19.8	1.8	1.1	2.3	3.4	2.1
	Peel	230	110	340	2.1	87.1	380	467	4.4
	whole	34.3	17.2	51.5	2	9.6	39.7	49.3	4.1
Red Lasoda	flesh	4.4	2.8	7.2	1.6	1.4	4.3	5.7	3.1
	Peel	134	96.6	231	1.4	10.5	24.83	35.3	2.4
	whole	16	11.2	27.2	1.4	2.2	6.1	8.3	2.8
Russet Burbank	flesh	25.9	21.4	47.3	1.2	11.1	56.5	67.6	5.1
	Peel	182	103	285	1.8	6.6	67.8	74.4	11.8
	whole	36.3	26.8	63.1	1.4	10.8	57.3	68.1	5.3
Russet Norkota	flesh	0.74	0.54	1.3	1.4	0.2	0.8	1	4
	Peel	48.1	23	71.1	2.1	33.6	129	163	3.9
	whole	4.8	2.5	7.3	1.9	3	11.9	14.9	4
Shepody	flesh	1.8	1.1	2.9	1.7	2.2	9.1	11.3	4.1
	Peel	282	147	429	1.9	44	299	343	6.8
	whole	25.1	13.2	38.3	1.9	5.6	33.1	38.7	5.9
Snowden	flesh	91.5	56.5	148	1.7	0.8	0.8	1.7	1
	Peel	372	171	543	2.2	54.2	96.3	150	1.8
	whole	119	67.9	187	1.8	5.8	10.2	16	1.8

6.2.4.1 Ratio of α-chaconine to α-solanine

The ratios of α-chaconine to α-solanine for the potato samples as seen in Table 6.2 ranged from 1.2 to 2.6. The ratio for peel, generally in the range of about 2, was much higher than for flesh with values near about 1.5. Since, as mentioned earlier, α-chaconine is more toxic than α-solanine, it is desirable to have this ratio as low as possible. We can only speculate about possible reasons for the wide variations in these ratios. Since the two glycoalkaloids, which share the common aglycone solanidine but not the same trisaccharide side chain (Figure 6.1), appear to be synthesized via distinctly different (discrete) biosynthetic channels (Choi et al., 1994), it is possible that the rates of biosynthesis of the two glycoalkaloids in the different channels are cultivar-dependent. Another possible rationalization for the varying ratios is that the rate of metabolism of the two glycoalkaloids is also cultivar-dependent. These considerations imply that alteration of the genes encoding enzymes involved in the biosynthesis of α-chaconine and/or α-solanine could have unpredictable results.

6.3 Calystegine Alkaloids

Calystegines are polyhydroxylated nortropane alkaloids present in potatoes (Richter et al., 2007; Kvasnicka et al., 2008). These water-soluble alkaloids were first discovered in 1988 from transformed root cultures of the non-food plant *Calystegia sepium* (Tepfer et al., 1988). Their structures were elucidated in 1990 (Goldmann et al., 1990). Since then, they have been found several other plant families, including the Solanaceae; specifically in *Solanum melongena* (eggplant) and *Solanum tuberosum* (potato), reported to contain calystegine A_3 and calystegine B_2 shown in Figure 6.3 (Nash et al., 1993; Asano et al., 1997; Keiner and Dräger, 2000; Bekkouche et al., 2001; Keiner et al., 2002). At least eight calystegines are currently known and many exhibit potent specific inhibition of glycosidases that are universally required for normal cell function. These polyhydroxylated alkaloids act as sugar mimics and inhibit glycosidases because of a structural resemblance to the sugar moiety of the natural substrate (Asano et al., 2000). Although no human toxicity data for calystegines have been reported,



calystegine A_3

calystegine B_2

Figure 6.3: Structures of potato calystegines.

polyhydroxylated alkaloids are reported to have therapeutic effects in the treatment of cancer, diabetes, bacterial and viral infection, and to stimulate the immune system (Asano et al., 2001; Watson et al., 2001). Biosynthetically, calystegines appear to be derived from the tropane alkaloids atropine and scopolamine also present in some Solanaceae plants such as *Datura stramonium* (Jimsonweed) (Dugan et al., 1989; Friedman and Levin, 1989; Keiner et al., 2000).

6.3.1 Methods

We validated and improved a GC-MS method to measure the two major potato calystegines A_3 and B_2 in freeze-dried potato peel and potato flesh samples (Friedman et al., 2003c). We used this method to analyze the same potatoes that we previously analyzed for glycoalkaloids (Table 6.2).

6.3.1.1 Extraction and isolation of hydrophilic alkaloid fractions

The procedure was adapted from that of Nash et al. (1993). Weighed samples (1.0 g) of powdered potato flesh and peel were stirred at room temperature for 24 h with methanol/water (4:1, v/v, 25 mL). Each sample was vacuum filtered through a pad of Celite diatomaceous earth to remove solids. The filtrate was concentrated to ~3 mL by rotary evaporation at 45°C. The residue was transferred quantitatively to a 10-mL beaker with deionized water and the pH adjusted to 4.0 with HCl. The more or less cloudy solution was introduced directly onto a 150 mm long × 12 mm diameter bed of cation-exchange resin (Dowex AG 50W × 8, Bio-Rad, Hercules, CA) at a flow rate of ~1 mL/min. After the column had been rinsed at the same flow rate with 55 mL of deionized water (~3 bed volumes), 0.5% NH_4OH (55 mL) was introduced and the alkaline eluent was collected and concentrated to 2–6 mL by rotary evaporation at 45°C. The resulting solution was transferred quantitatively to a 10-mL volumetric flask and made up to 10 mL with deionized water from which 1.0-mL aliquots were transferred to 4-mL borosilicate glass screw cap vials, frozen in liquid nitrogen and freeze-dried.

6.3.1.2 Preparation of trimethylsilyl (TMS) ether derivatives

To each vial was added dry pyridine (45 μL), N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) (Pierce, Rockford, IL) (45 μL), and as internal standard 10 μL of a solution of 250 μg of perseitol (Aldrich) in 50 μL each of pyridine and MSTFA that had been previously warmed for 1 h at 100°C. The vials were then heated for 1 h in a Reacti-Therm block heater (Pierce).

6.3.1.3 GC-MS analysis

The derivatized samples were analyzed on a Hewlett-Packard 5890 series II GC (helium carrier gas) coupled to a Hewlett-Packard 5971 mass-selective detector (MSD). A 60 m, 0.32 mm i.d., 0.25-im film, SE-30 fused silica capillary column (J&W Scientific, Folsom, CA) was installed in the GC, and an on-column injector (SGE model OC1-3) held at ambient temperature was fitted to the column inlet. Samples (0.5 μL) were injected directly into the column held at 105°C

for 0.2 min. The column was ramped at 30°C/min for 0.5 min, programmed from 120 to 300°C at 10°C/min, and held at the final temperature for 10 min. The MSD was operated at 70 eV in the EI mode with scans taken every 1.5 s from 75 to 600 amu. A postinjection delay of 7.0 min was set to allow solvent and derivatizing agent to elute before mass spectral data acquisition began. Retention times and mass spectra of calystegine standards confirmed the presence of the trihydroxy nortropane alkaloid, calystegine A_3, and the tetrahydroxy nortropane alkaloid, calystegine B_2, as TMS ethers in all eight of the potato cultivars examined. The amounts of the two alkaloids were calculated by comparison of the integrated total ion current peak areas with the peak area of the internal standard, perseitol-TMS.

6.3.2 Results

Figure 6.4 illustrates the separation on GC-MS total ion chromatograms of calystegines A_3 and B_2 extracted from potato powders. Results of the GC-MS analyses for calystegines A_3 and B_2 are shown in Table 6.2. For whole potatoes, there is a 12-fold variation from lowest

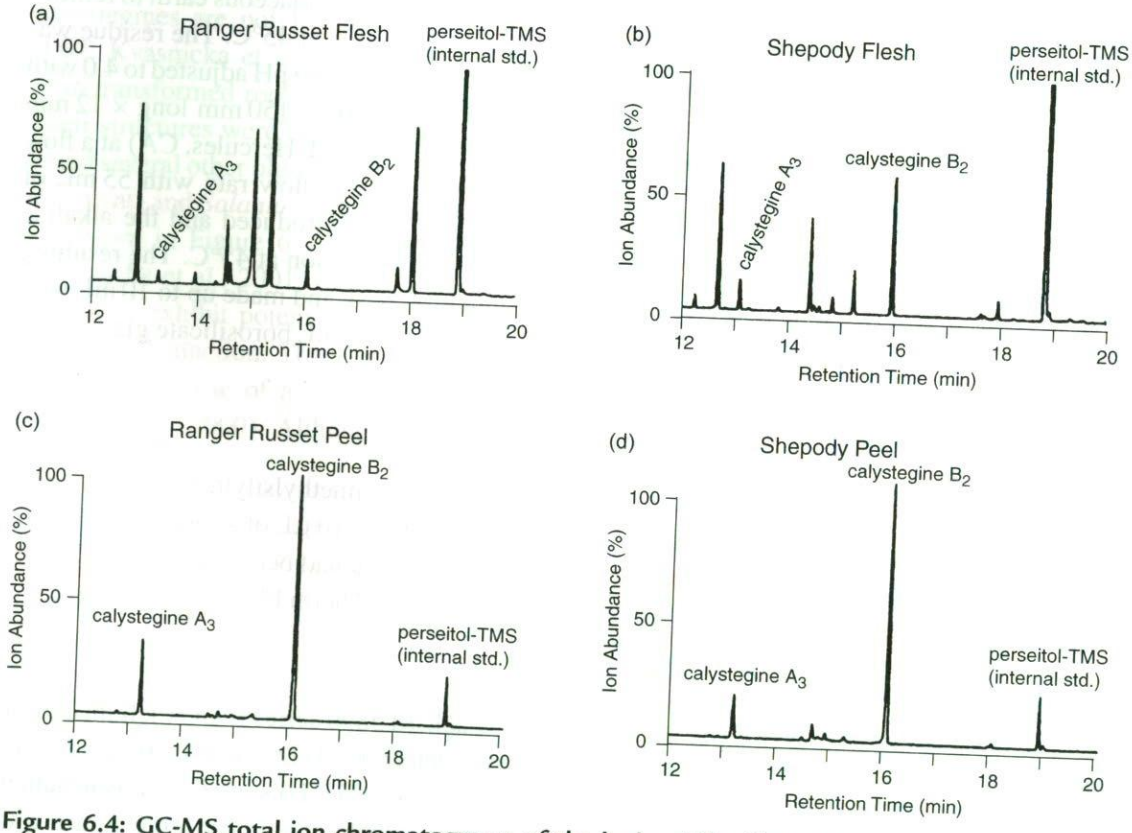


Figure 6.4: GC-MS total ion chromatograms of the hydrophilic alkaloid fraction extracted from freeze-dried potato flesh and peel.

to highest values for A_3 and a 15-fold variation for B_2. For the sum of the two calystegines ($A_3 + B_2$ column), there is a 13-fold variation from lowest to highest values. For calystegine B_2/A_3 ratios, there is a 3-fold variation from lowest to highest values. It is also instructive to calculate the following ratios of total glycoalkaloid to total calystegine content for whole potatoes: Russet Norkota, 0.49; Atlantic, 0.49; Russet Burbank, 0.93; Shepody, 0.99; Ranger Russet, 1.0; Red Lasoda, 3.3; Dark Red Norland, 4.5; Snowden, 11.7. The biosynthesis of glycoalkaloids seems to parallel that of calystegines in some varieties but not in others. The concentrations of both calystegines and glycoalkaloids are greatest in the peel. Relative levels in the flesh and peel vary widely among the cultivars evaluated. That the glycoalkaloid/calystegine ratio also varies implies that the synthesis of these two classes of secondary metabolites may be under separate genetic control. Because there is some correlation between their levels, they may respond similarly to some stress conditions. Moreover, since the individual calystegine isomers differ in their biological activities (Asano et al., 1997; Watson et al., 2001), both their ratios as well as total amounts present in different potato cultivars may be important in assessing the role of calystegines in the diet. Since the biological activities as well as the roles in host-plant resistance of both glycoalkaloids and calystegines could be interrelated, there is a need to define the levels of both glycoalkaloids and calystegines in different potato cultivars and to study individual and combined effects in animals and humans. Possible therapeutic applications of high-calystegine potato diets also merit study.

6.4 Phenolic Compounds

Phenolic compounds are secondary plant metabolites found in potatoes and other plants (reviewed in Friedman, 1997; Mattila and Hellström, 2007). In the plant, phenolic compounds function beneficially to defend against invading pathogens, including bacteria, fungi, and viruses. They also, however, participate in enzyme-catalyzed browning reactions that may adversely affect color, flavor, and nutritional quality of potatoes. Antioxidative phenolic compounds show promise as health-promoting phytochemicals as they have been shown to exhibit beneficial antimutagenic, anticarcinogenic, antiglycemic, anticholesterol, and antimicrobial properties. These considerations suggest the need for accurate analysis of phenolic compounds in potato leaves, stems, and tubers and in processed potato products.

Figure 6.5 shows the structures of *trans*-cinnamic acid and four cinnamic acid derivatives (phenolic compounds) reported to be present in potatoes. Because potatoes are one of our major food plants, we validated with the aid of HPLC and LC/MS the content and distribution of antioxidative phenolic compounds in parts of the potato plant, in potato tubers, in the peel and flesh of tubers, in potatoes sold commercially in Korea and the United States, and in home-processed potatoes. The following discussion, based on our own studies, is followed by a brief overview of analytical methods for potato phenolic compounds by other investigators.

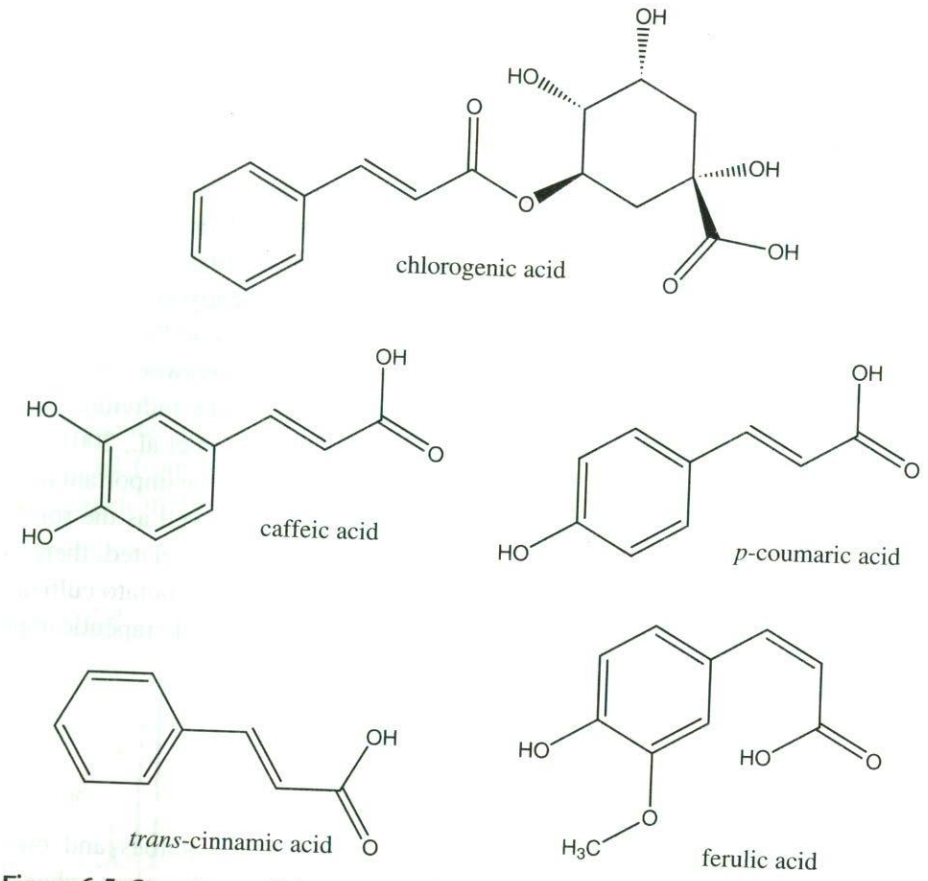


Figure 6.5: Structures of cinnamic acids derivatives commonly found in potatoes.

6.4.1 Analysis

Reported analytical methods for potato phenolics include HPLC (Percival and Baird, 2000; Tudela et al., 2002; Verde Méndez Cdel et al., 2004; Shakya and Navarre, 2006; Mattila and Hellström, 2007; Reddivari et al., 2007a), capillary electrophoresis (Fernandes et al., 1996), colorimetry/spectrophotometry (Friedman et al., 1989; Dao and Friedman, 1992; Griffiths et al., 1992; Dao and Friedman, 1994; Percival and Baird, 2000; Kanatt et al., 2005; Stratil et al., 2006), and GC/MS (Tisza et al., 1996). The HPLC and LC/MS methods we previously used to determine glycoalkaloids and vitamin C in potatoes (Friedman et al., 2003c; Han et al., 2004) were adapted to the analysis of potato phenolic compounds (Im et al., 2008). Figure 6.6 shows a chromatogram of potato flesh before (a) and after (b) spiking. Figure 6.7 shows a LC/MS chromatogram of potato peel, monitoring absorbance at 280 nm, 340 nm, and the TIC.

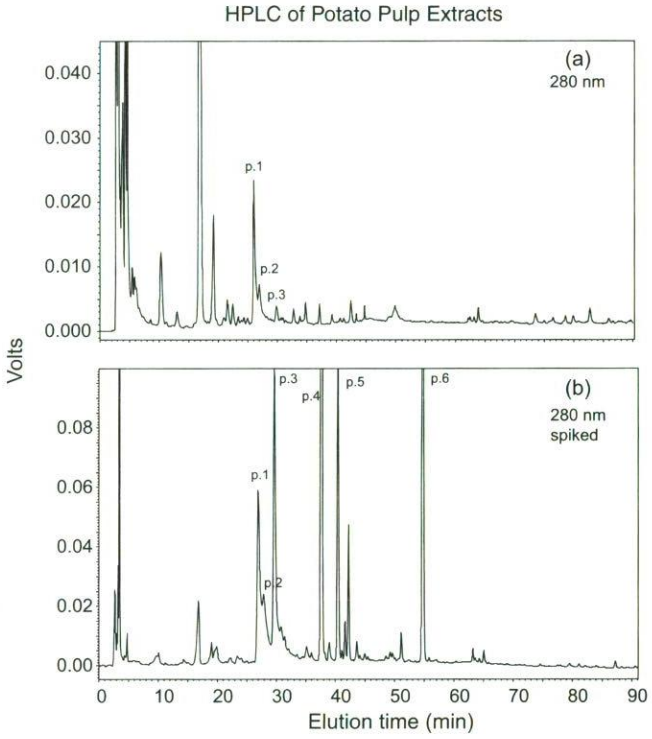


Figure 6.6: HPLC chromatogram of the extract from Superior potato flesh (a) and of the same extract spiked with standards (b). Identification; p.1, chlorogenic acid; p.2, chlorogenic acid isomer; p.3, caffeic acid; p.4, p-coumaric acid; p.5, ferulic acid; p.6, t-cinnamic acid. Column, Inertsil ODS-3 v (5 μm, 4.0 x 250 mm); flow rate, 1.0 mL/min; column temperatures, 20°C; mobile phase, acetonitrile:0.5% formic acid (gradient mode); detector, UV at 280 nm.

6.4.1.1 Extraction of phenolic compounds from peels and flesh of potato tubers

The potato tubers were each peeled to a depth of 2–3 mm. Fresh peel weights amounted to 21.1–23.9% of the total weight of the potatoes. Fresh peel and flesh were then cut with a knife into 4-mm-thick slices. Samples (10 g) of peel and flesh from each potato tuber were then placed into a 250-mL flask with a reflux condenser to which was added 50 mL of 80% ethanol, followed by heating at 80°C for 10 min. After homogenization in a Waring blender, the mixture was again transferred to a flask for re-extraction, followed by centrifugation at 12 000 ***g*** for 15 min at 5°C. The residue was extracted twice with 20 mL of 80% ethanol and centrifuged. The combined extracts were made up to 100 mL with 80% ethanol. This solution (10 mL) was evaporated under reduced pressure at 20°C and the residue was dissolved in 80% ethanol (1 mL) and centrifuged. The supernatant (20 μL) was used for HPLC.

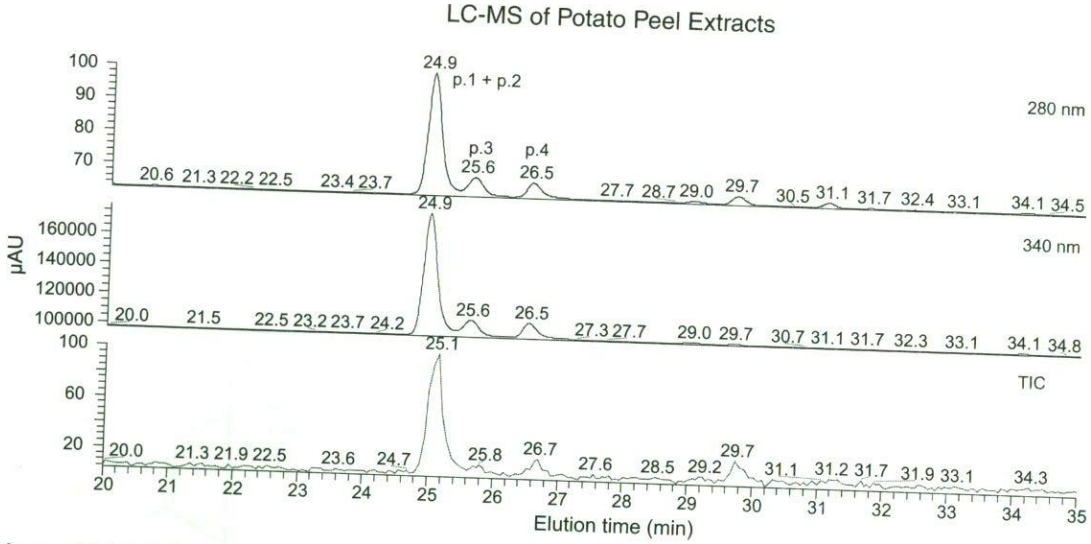


Figure 6.7: LC-MS chromatograms of an extract of Superior potato peel monitored at 280 nm, 340 nm, and TIC. Column: Inertsil ODS-3 (3 μm, 4.0 x 150 mm). Flow rate: 0.2 mL/min. Column temperature: 30°C. Mobile phase: acetonitrile: 0.5% formic acid (gradient mode).

6.4.1.2 *HPLC analysis*

HPLC analysis was carried out on a Hitachi liquid chromatograph model 665-II equipped with a Shimadzu UV-VIS detector (Model SPD-10Avp, Kyoto, Japan) set at 280 nm and 340 nm. Column temperature was controlled with a Shimadzu CTO-10Asvp Thermometer. Chromatogram peak areas were integrated with a Hitachi D-2500 chromato-integrator. An Inertsil ODS-3v column [5 μm, 4.0 × 250 mm (GL Science Inc., Tokyo, Japan)] was used to analyze the phenolic acids. The mobile phase of the (A/B) gradient was (A) acetonitrile and (B) 0.5% formic acid. The content of acetonitrile in the solvent was increased as follows: 5% (0–5 min); 18% (30 min); 70% (90 min); 5% (120 min). The flow rate was 1 mL/min at a column temperature of 20°C. Three separate analyses were carried out with each sample. Plots of concentration versus peak areas (calibration plots) were linear at the concentration range of 8.0 (LOD)–300 ng for ferulic acid; 4.7–400 ng for caffeic; 2.0–400 ng for *trans*-cinnamic acids; 3.2–600 ng for coumaric acid; and 16.5–800 ng for chlorogenic acid. Percent recoveries of spiked samples were as follows (n = 3): trans-cinnamic acid, 95.2; chlorogenic acid, 97.2; p-coumaric acid, 102.0; ferulic acid, 102; and caffeic acid, 107.

6.4.1.3 *LC-MS/MS*

Liquid chromatography/mass spectrometry analyses were performed with an ion trap mass spectrometer (LCQ, Thermo Fisher Scientific Inc., MA) equipped with an HPLC system (Agilent, CA; Model 1100) connected with a diode-array detector (DAD, G1315A). The sample solution (1–5 μL) was applied on an Inertsil ODS-3 column (2.1 × 150 mm, 3 μm, GL

Sciences Inc., Tokyo, Japan) and was separated using gradient solvent system at the flow rate of 200 μL/min. The content of acetonitrile in 0.5% formic acid was increased as follows: 5% (0–5 min); 18% (30 min); 70% (90 min). The LC eluate was introduced into the mass spectrometer after 3 min of the sample injection. The MS/MS experiments were carried out in the negative-ion modes. The parameters were optimized using a standard chlorogenic acid solution by mixing the mobile phase (50% acetonitrile) eluted from the LC system as follows: ESI spray voltages; 4.5 kV (negative-mode); capillary temperature, 50°C; capillary voltages, −42 V (negative-mode); sheath gas (nitrogen) flow rate, 64 (arbitrary unit); auxiliary gas flow rate, 55 (arbitrary unit); tube lens offset voltages, −15 V; multipole 1 offset voltages, 1.0 V; multipole 2 offset voltages, −7.0 V; intermultipole lens voltages, 14 V. Helium was used as collision gas and the relative collision energy was set at 40% for MS/MS and MS3 experiments over a selected mass window of 2 Da. Mass selection of the analyte by m/z was followed by fragmentation and analysis of the fragments.

6.4.2 Identification

Structural identification of individual phenolic compounds in extracts was performed by associating the HPLC peak of each compound with the corresponding UV (Figure 6.8) and mass spectrum (Figure 6.9). The HPLC chromatograms (Figure 6.6) demonstrate the presence of caffeic, chlorogenic acid (5-caffeoylquinic acid), and a chlorogenic acid isomer with the same molecular weight as chlorogenic acid in the potato extracts. We saw no evidence for the presence of *p*-coumaric and ferulic acids in these extracts.

6.4.3 Discussion

Chlorogenic acids (CGA) are a family of esters formed between *trans*-cinnamic acids and (-)-quinic acid (Clifford et al., 2003). At least three isomeric forms of chlorogenic acid may be present in potatoes: 3-, 4-, and 5-caffeoylquinic acids (Fernandes et al., 1996; Friedman, 1997). UV light induces the isomerization of naturally occurring *trans*-chlorogenic isomers to the *cis* form (Clifford et al., 2008). The HPLC and LC/MS data of the present study indicate the presence of two isomeric forms, 5-caffeoylquinic acid for which we had a standard, and another isomer with the same molecular weight for which we do not have a standard. The LC/MS patterns illustrated do not differentiate between the two isomers. In analogy with the observation by Fernandes et al. (1996) that the 5- and 4-caffeoylquinic isomers were present in quantifiable amounts in potatoes in a ratio of 8.5:1, it is likely that the smaller of the two isomer peaks is probably 4-caffeoylquinic acid. Table 6.3 shows the levels of phenolics we found in potato, leaves, and stems. The data also show that chlorogenic acid and its isomer constituted ~96–98% of the total phenolic content and that the total in flowers was 2.74 times greater than in leaves and 58.5 times greater than in stems. Although we do not know the reason for the high levels in the flowers, a likely explanation is that the high amounts are needed to protect the flowers against attacks by phytopathogens.

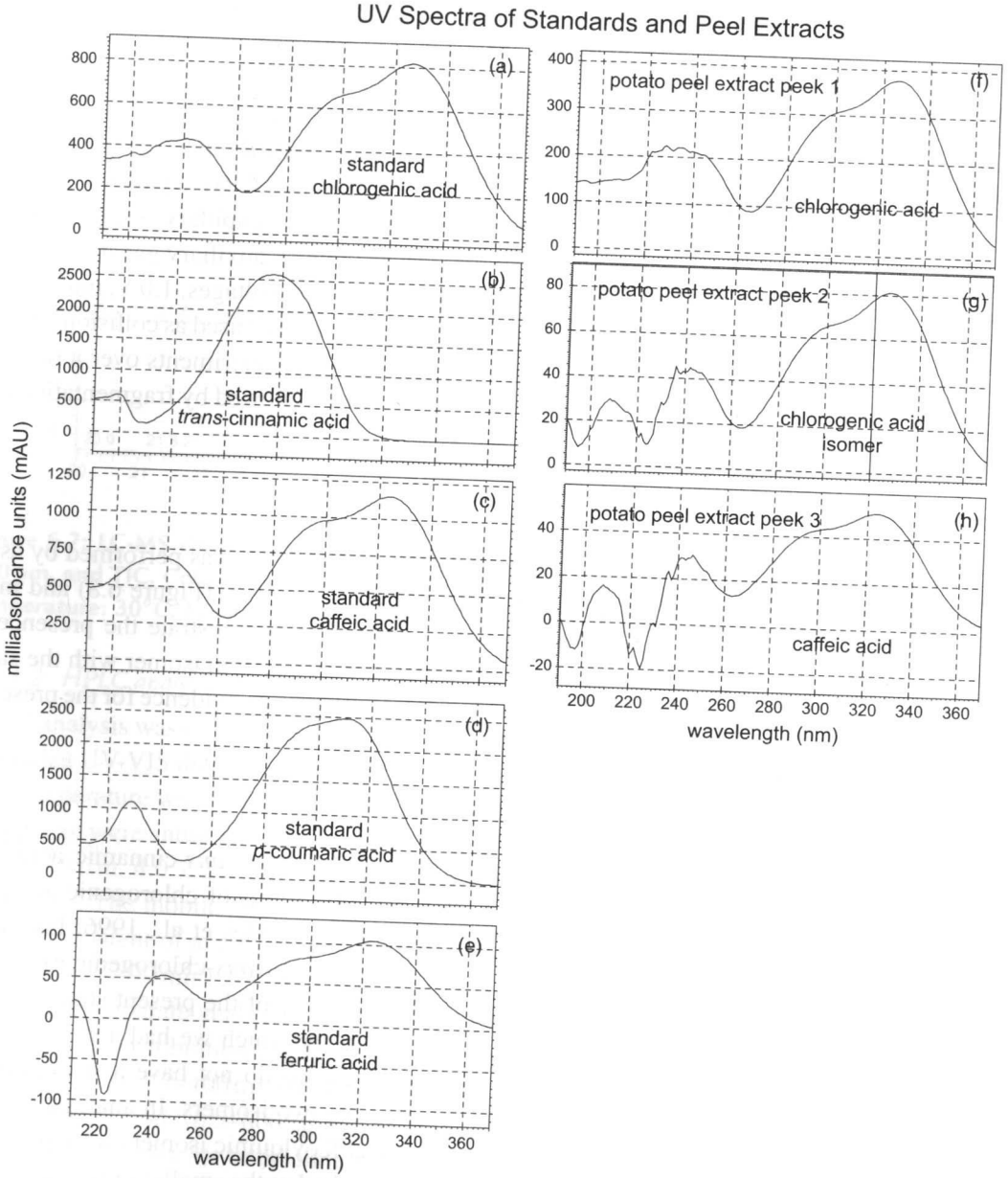


Figure 6.8: UV spectra of standard chlorogenic acid (a); *trans*-cinnamic acid (b); caffeic acid (c); *p*-coumaric acid (d); and ferulic acid (e). The spectra of peaks 1 (chlorogenic acid) (f), peak 2 (chlorogenic acid isomer) (g), and peak 3 (caffeic acid) (h) were determined with HPLC fractions isolated from extracts of Superior potato peel.

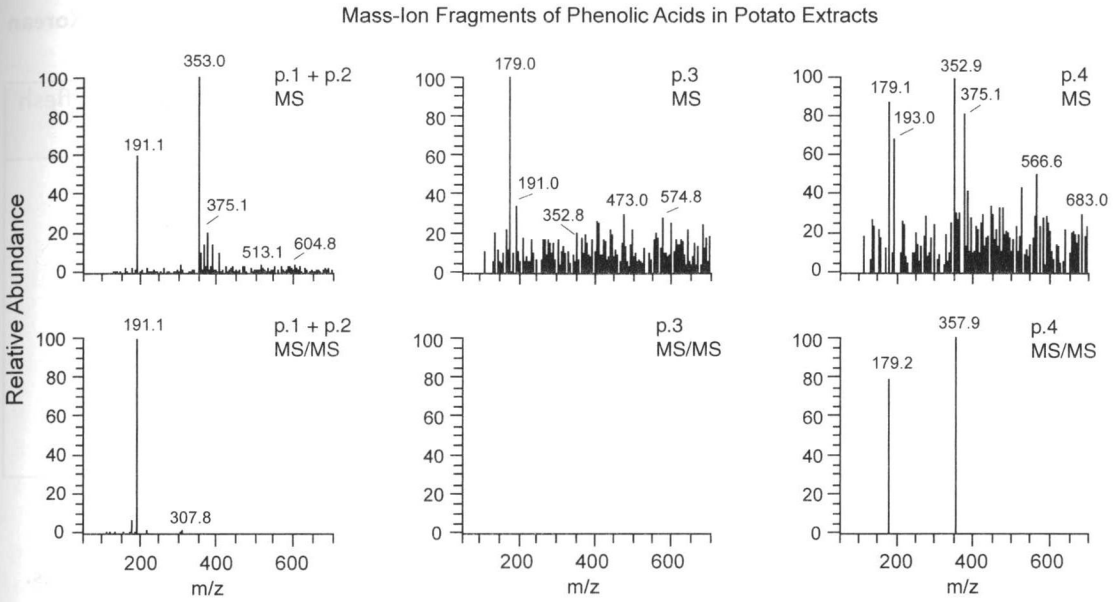


Figure 6.9: MS and MS/MS (negative ion mode) of peaks 1 and 2 (chlorogenic acid and its isomer), peak 3 (caffeic acid) and peak 4 (chlorogenic acid isomer) from isolated HPLC chromatograms of potato extracts.

Table 6.3: Distribution (in mg/100g fresh wt) of phenolic compounds in three parts of superior potato plants

Plant part	Chlorogenic acid	Chlorogenic acid isomer	Caffeic acid	Total
Flower	424	189	13.5	626
Leaf	14.8	13.3	1.2	29.3
Stem	4.2	6.1	0.4	10.7

Table 6.4 shows the distribution of the three phenolics in the peel and flesh of five commercial potato varieties grown in Korea. Noteworthy is the large variation in the ratio of peel to flesh levels ranging from 2.55 for the Jasim to 21.1 for the Jowon potatoes.

Table 6.5 shows the distribution of the three phenolics in the peel and flesh of the Korean Superior potato variety available in four sizes: large, medium, small, and very small. The data indicate that the size of the potato does not seem to influence the total phenolic content, except that the ratio of peel to flesh for the very small potatoes (7.95) is about one half the corresponding ratios of the other three potatoes. These results indicate that the distribution of phenolic compounds between peel and flesh varies widely among different potato varieties. They also suggest that

Table 6.4: Content (in mg/100 g fresh wt) of phenolic compounds in the peel and flesh of Korean potato varieties

Potato variety	Potato section	Chlorogenic acid	Chlorogenic isomer	Caffeic acid	Total	Ratio: peel/flesh
Jasim	Peel	34.0	6.8	1.2	42.1	2.6
	Flesh	12.0	4.4	0.11	16.5	
Atlantic	Peel	4.4	1.8	0.96	7.2	14.6
	Flesh	0.35	0.13	0.01	0.5	
Jowon	Peel	10.2	3.0	0.7	13.9	21.1
	Flesh	0.45	0.17	0.04	0.66	
Superior	Peel	8.7	0.99	1.2	10.9	20.2
	Flesh	0.47	0.06	0.01	0.54	
Jopung	Peel	4.9	1.3	0.39	6.6	7.6
	Flesh	0.57	0.26	0.02	0.85	

Table 6.5: Effect of potato size on the phenolic acid content of the peel and flesh of potatoes. Listed values in mg/100 g fresh wt

Superior potato size	Potato part	Chlorogenic acid	Chlorogenic acid isomer	Caffeic acid	Total	Ratio peel/flesh
Large	Peel	7.4	0.92	1.1	9.3	15.8
	Flesh	0.53	0.04	0.02	0.59	
Medium	Peel	8.7	0.99	1.2	10.9	20.2
	Flesh	0.5	0.06	0.01	0.54	
Small	Peel	5.3	0.61	1.6	7.5	16.7
	Flesh	0.39	0.05	0.01	0.45	
Very small	Peel	8.3	1.0	0.04	9.4	7.9
	Flesh	1.0	0.11	0.04	1.2	

consumers and potato processors can select from available potato varieties those with high, intermediate, or low amounts of phenolic compounds. Additional studies revealed that the total phenolic content of the vertical slices ranged from 0.79 to 2.49 mg/100 g fresh wt, a 3.15-fold variation from highest to lowest. The corresponding range for the horizontal slices was from 0.84 to 6.58 mg/100 g fresh wt, a 7.83-fold variation from highest to lowest. These results suggest that it may be possible to select, depending on need, slices with high or low amounts of phenolic compounds for the preparation of potato-based foods. Such selection should take into account possible variability of phenolic content in different tubers of the same cultivar.

Table 6.6 lists the phenolic acid content of 25 potato powders prepared by lyophilization of commercial potatoes with unknown history. For the dry lyophilized powders, the data show that chlorogenic acid levels (in mg/100 g wt) ranged from 3.28 for Kenebec potatoes to 637 for

Table 6.6: Content of phenolic compounds of freeze-dried powders prepared from fresh potatoes sold in the United States

Potato variety	Chlorogenic acid	Chlorogenic acid isomer	Caffeic acid	H_2O in fresh tubers (%)	Total phenolics (mg/100 g)	
					Dry tubers	Fresh tubers
Kennebec	3.28	0.34	0.47	75.0	4.09	1.03
Russet, baking, batch 1	18.2	1.0	1.8	79.5	21.0	4.3
White, large, batch 2	14.5	4.9	2.0	83.4	21.4	3.6
Yukon Gold, grade "A", large	14.3	4.7	4.6	76.9	23.6	5.5
Yukon Gold, grade "B", medium, batch 1	19.0	6.0	5.0	75.5	30.0	7.4
White, large, batch 1	21.6	6.7	2.8	79.9	31.2	6.3
Russet, baking, batch 2	25.9	10.7	2.5	79.6	39.1	8.0
Yukon Gold, grade "C", small, batch 1	26.7	7.5	7.9	82.3	42.1	7.5
Yukon Gold, grade "C", small, batch 2	26.0	12.3	5.3	82.7	43.6	7.5
Red, medium, organic	35.0	8.0	4.0	82.3	47.0	8.3
White, medium	34.1	12.8	6.0	79.4	53.0	10.9
Yukon Gold, grade "B", medium, batch 2	35.6	8.9	9.3	82.4	53.8	9.5
Butterball Creamer, organic, German	35.95	13.4	7.1	80.5	56.5	11.0
White Creamer, small	41.9	10.4	9.8	83.4	62.1	10.3
Ruby Red Crescent	49.5	16.4	6.5	78.9	72.4	15.3
Red, grade "A", large, batch 1	56.4	17.0	7.9	80.9	81.3	15.5
Red, grade "A", large, batch 2	64.5	16.7	10.4	83.1	91.7	15.5
Red Creamer Marble	65.6	37.2	1.1	84.2	103.9	16.4
Red, grade "C", small	73.1	22.4	15.2	81.3	110.7	20.7
Fingerling, Ozette, batch 3	92.3	44.9	10.4	79.5	147.6	30.3
Purple, large	108.6	37.9	5.3	75.3	151.7	37.5
Fingerling, Ozette, batch 1	104.6	49.7	13.8	80.0	168.0	33.6
Fingerling, Ozette, batch 2	113.5	41.9	12.7	78.5	168.0	36.1
Fingerling French	203.0	69.7	7.8	79.3	280.5	58.0
Purple Peruvian	637.3	90.5	29.3	77.3	757.0	171.8

Purple Peruvian potatoes, a 194-fold variation from lowest to highest value. The corresponding range for the chlorogenic acid isomer was from 0.34 to 90.5, a 266-fold variation; and for caffeic acid, from 0.47 to 29.3, a 62.3-fold variation. Total amounts of phenolic compounds for the dry powders ranged from 4.09 to 757, a 185-fold variation; and for fresh tubers from 1.03 to 172, a 167-fold variation. The cited observations demonstrate wide variation both in individual and total phenolic acid content of commercial potatoes. The red- and purple-colored potatoes contained the highest amounts of phenolic compounds. Our data show that commercial potatoes evaluated differ widely in their content of phenolic acids. There is a need to determine possible relationships between phenolic content and health-promoting potential of different commercial potato varieties (Rodriguez de Sotillo et al., 1998; Rauha et al., 2000; Friedman et al., 2003b; Kanatt et al., 2005; Rivera-Carriles et al., 2005; Nara et al., 2006; Huang et al., 2007).

6.4.3.1 *Home processing*

The contents of soluble phenolic acids in raw potato peels determined by HPLC varied from 23 to 45 mg/100 g fresh wt. Boiled peels contained lower amounts (Mattila and Hellström, 2007). Cooking of potatoes and other vegetables in small amounts of water retained most of the phenolic compounds (Andlauer et al., 2003). Steamed potato strips retained 42% of initial chlorogenic acid content and frying, 24% (Tudela et al., 2002). Similar decreases were observed in the content of caffeic acids following exposure of the strips to home-processing conditions. Figure 6.10 shows the effect of several home-processing methods on polyphenol content in potatoes (Im et al., 2008). Chlorogenic acid loss is greatest with boiling in 3% salt, suggesting

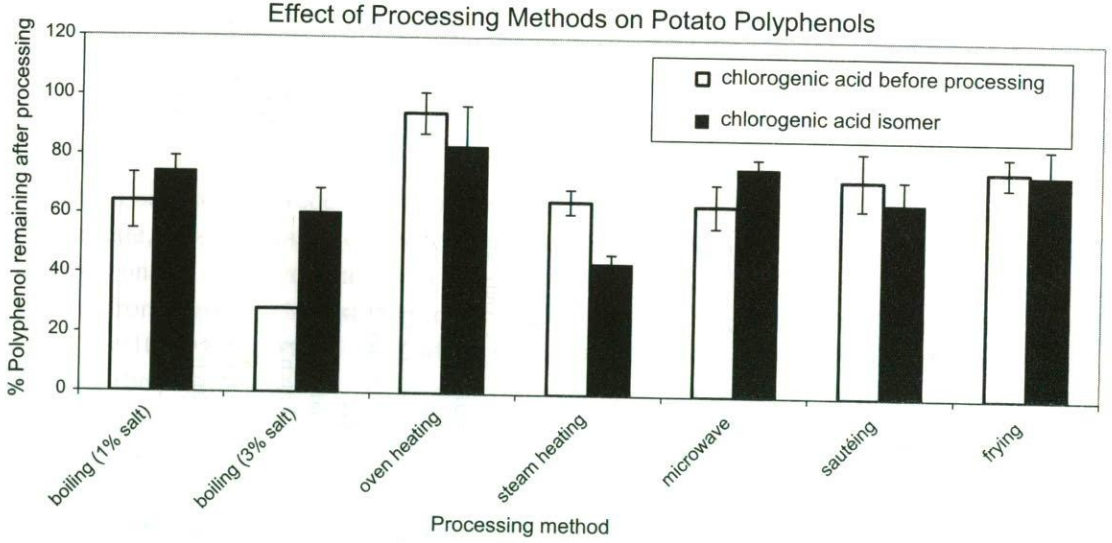


Figure 6.10: Effect of processing method on loss of phenolic compounds of Superior potato flesh. Values are averages from three separate determinations ± SD versus matched controls.

that the compound is leaching into the water. Oven heating provides the best retention of both compounds.

6.4.4 Other studies

We previously measured the chlorogenic acid content of seven potato varieties by UV spectroscopy. Tuber values ranged from 9.6 to 18.7 mg/100 g fresh wt, and leaves harvested at different times from 132 to 242 mg/100 g fresh wt (Dao and Friedman, 1992, 1994; Friedman, 1997). The chlorogenic acid content of 145 mg/100 g freeze-dried potatoes determined by electrophoresis was similar to that determined by HPLC (154 mg/100 g) (Fernandes et al., 1996). No other phenolic compounds were detected in quantifiable amounts. These authors also reported that exposure of the tubers to light resulted in significant increases in chlorogenic acid content, confirming related observations by other investigators (Dao and Friedman, 1994; Griffiths et al., 1995; Percival and Baird, 2000). Storage can cause an accumulation of phenolics. Total phenolic acid content of potatoes (in mg/100 g fresh wt) grown in India increased from 50.6 to 83.7 during storage for 120 days (Mehta and Singh, 2004). Genetic modification induced both inadvertent (Defernez et al., 2004) and purposeful (Lukaszewicz et al., 2004) increases in the phenolic acid content in some potato varieties. However, there was no increase in the transgenic potato Spunta (El Sanhoty et al., 2004).

There is considerable variability of phenolics in potatoes. The average chlorogenic and caffeic acid content of five potato varieties (in mg/100 g fresh wt) grown in the Canary Islands ranged from 21.0 to 28.3 and from 0.73 to 1.12, respectively (Verde Méndez Cdel et al., 2004). Total phenolic acid content of 74 potato cultivars grown in the Andes of South America ranged from 1.12 to 12.37 mg of gallic acid equivalents/g dry wt, an 11-fold variation from lowest to highest value (Andre et al., 2007). The total phenolic acid content of specialty potato selections grown in Texas ranged from 221 μg to 1252 μg chlorogenic acid equivalent, a 5.7-fold variation from lowest to highest value (Reddivari et al., 2007a). Purple flesh selections had the highest amounts, followed by red flesh and yellow selections. Other studies found that the caffeic acid content of different potato cultivars varied widely, ranging from 0.3 to 3.6 mg/100 g in tubers and from 18.8 to 28 mg/100 g in peels (Dao and Friedman, 1992; Dao and Friedman, 1994; Mattila and Kumpulainen, 2002; Mattila and Hellström, 2007).

The cited observations suggest that it is possible to identify potato cultivars with low or high phenolic acid content for human use and to select processing conditions that minimize losses of phenolic compounds. In summary, the methods we developed and used to determine the content and distribution of phenolic compounds in potato plant flowers, leaves, and tubers, in the peel and flesh parts of potato tubers, and in freeze-dried and processed commercial potatoes merit application in numerous studies designed to assess the role of potato phenolic compounds in host-plant resistance, plant breeding, plant molecular biology, food chemistry, nutrition, and medicine. The described wide distribution of phenolic compounds in different commercial

potato varieties and on changes in phenolic compound content during home processing of potatoes may also help consumers to select, depending on need, potatoes with low or high levels of health-promoting phenolic compounds, to use processing conditions that minimize their degradation, and to control enzymatic browning reactions (Molnar-Perl and Friedman, 1990; Friedman and Bautista, 1995) that are reported to cause undesirable discolorations and to damage nutritional quality.

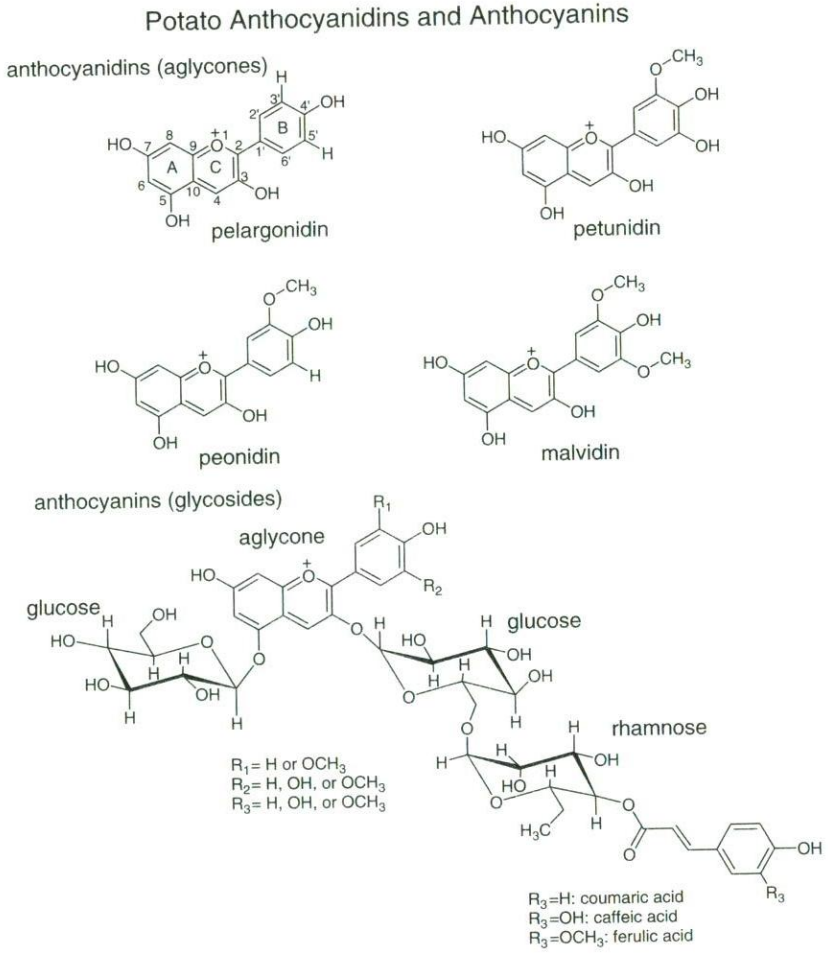
6.5 Anthocyanins

In this section, we will briefly review the chemistry, plant physiology, processing effects, composition, and biological properties of potato anthocyanins. Anthocyanins are widely distributed among plants. They are pigments responsible for many of the blue, red, and violet colors in plants. Anthocyanins have an array of health-promoting benefits primarily acting as antioxidants through a variety of mechanisms (Kong et al., 2003). They also have the potential to be used in the food-processing or the pharmaceutical industry as natural alternatives to synthetic antioxidants, stabilizers, and colorants (Wrolstad et al., 2001; Andre et al., 2007). For instance, because extracts from red- and purple-flesh potatoes at pH 3 showed similar hues as the dye FD&C Red #40, they have the potential to replace that colorant (Giusti and Wrolstad, 2003; Reyes and Cisneros-Zevallos, 2007). Because they are natural and health-giving, they are likely to gain wide consumer and regulatory acceptance.

6.5.1 Chemistry

Figure 6.11 depicts the structures of anthocyanins found in colored potatoes. Anthocyanins are 3-mono- or 3,5-diglucosides of anthocyanidins (aglycones): primarily pelargonidin, petunidin, malvidin, and peonidin in potatoes. Their chemical structure governs color, tinctorial strength, and stability. Substitutions can affect the tertiary and quaternary formations of the molecule, making the chromophore more or less susceptible to hydration, which causes color loss. Acetyl and cinnamoyl moieties are often attached to the glucosyl side chain (Brouillard et al., 2003). The presence of caffeyl residues in the anthocyanin structure not only confers color stability but also allows color diversification (Dangles et al., 1993). Acylation with cinnamic acid shifts colors to purple, increases hydration and antioxidant capacity, and decreases visual detection thresholds (Stintzing et al., 2002). Sugar substituents in the 3- and 5-position also affect these parameters. Molar absorptivities of anthocyanins ranged from 15 600 to 39 590 for pelargonidin-3-glucoside (pg-3-glu) and pg-3-rutinoside-5-glucoside acylated with p-coumaric acid, respectively. Small differences in chemical structure appear to have large effects on color and tinctorial strength of anthocyanin extracts.

Stability is also affected by pH, light, heat, and mechanical stress. Colors change depending on pH and on protonation and hydration reactions during storage. The most stable form, the flavylium cation, predominates at low pH (Torskangerpoll and Andersen, 2005). Stability is



R_1=H, R_2=H, R_3=H: pelargonidin 3-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=H, R_3=H: peonidin 3-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside; peonarin

R_1=OCH_3, R_2=H, R_3=OH: peonidin 3-[6-*O*-(4-*O*-*E*-caffeoyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OH, R_3=H: petunidin 3-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside; petanin

R_1=OCH_3, R_2=OH, R_3=OH: petunidin 3-[6-*O*-(4-*O*-*E*-caffeoyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OH, R_3=OCH_3: peonidin 3-[6-*O*-(4-*O*-*E*-feruloyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OCH_3, R_3=H: malvidin 3-[6-*O*-(4-*O*-*E*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OCH_3, R_3=OCH_3: malvidin 3-[6-*O*-(4-*O*-*E*-feruloyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

Figure 6.11: Structures of Anthocyanins found in potatoes.

also influenced by light and temperature (Reyes and Cisneros-Zevallos, 2007). Anthocyanins in extracts of red-flesh potatoes were more stable to thermal degradation at pH 3 than were corresponding extracts from purple-flesh potatoes (Reyes and Cisneros-Zevallos, 2007). The stability of anthocyanin extracts to pH <3 and thermal degradation at pH 3 followed first-order kinetics. Changes in lightness and hue followed zero-order kinetics, while changes in chroma followed first-order kinetics.

6.5.2 Plant physiology

Anthocyanins have multiple functions in the plant (Lukaszewicz et al., 2004). These include visual attraction of pollinators, acting as feeding deterrents, photoreceptors to protect against damage by UV radiation, and as chelators of metal ions (Hamouz et al., 2006, 2007; Lachman et al., 2008). Increasing anthocyanin content in crop plants may result in self-protection against biotic and abiotic stresses and may lead to health-promoting effects after consumption. Post-harvest wounding stress induced increases in both anthocyanins and total phenolic content of purple-flesh potatoes (Reyes and Cisneros-Zevallos, 2003). Slicing induced ~60% increases in total phenolics, including anthocyanin levels, with a parallel 85% increase of antioxidant capacity. The authors suggest that controlled post-harvest abiotic stresses could induce accumulation of antioxidants, thus enhancing the nutritional and health-promoting value of potatoes and other horticultural food crops. Slicing, used in the preparation of potato chips and French fries, could be used as a tool to boost the anthocyanin content to obtain healthier products. Use of such a tool would have to be weighed against any detrimental effects of polyphenol oxidase catalyzed reactions and glycoalkaloid production. Perhaps the processes could be uncoupled. Could the high phenolic content of wounded potatoes also protect against acrylamide formation in chips and fries (Friedman and Levin, 2008)? Anthocyanin content also correlated with potato tuber resistance to bacterial infection by *Erwinia carotovora* (Lorenc-Kukula et al., 2005) and *Pectobacterium cartovorum* (soft-rot) (Wegener and Jansen, 2007).

6.5.3 Genetic manipulation

It was possible to increase the anthocyanin content of transgenic potatoes by overexpressing genes that govern the formation of the enzymes dihydroflavonol 4-reductase (DFR), chalcone synthase (CHS), user and chalcone isomerase (CHI), which all catalyze the biosynthesis of anthocyanins (Lukaszewicz et al., 2004). The resulting increases in anthocyanin and phenolic acid content correlated with increases in antioxidant activities of the potato extracts, but less than expected, inferring that other processes may be at work. Stobiecki et al. (2003) created transgenic potato plants overexpressing and repressing enzymes involved in biosynthesis of flavonoids. Overexpression of DFR with in-sense orientation resulted in an increase in tuber anthocyanins, just as the antisense orientation resulted in decreased anthocyanins. The transformation of these potato plants was also accompanied by significant changes in glycoalkaloids, although changes were not dependent on flavonoid composition, except in the transgenic plants

containing the highest and lowest flavonoids, in which case there was a positive correlation. It appears that the changes in glycoalkaloids resulted not from the gene construct used for transformation on orientation of coding sequence, nor on the site of transgene incorporation, but from chromatin stressed upon transformation. The possibility of decreasing the concentration of toxic compounds in potato tubers while increasing flavonoids appears advantageous. Transgenic studies aimed at manipulating other aspects of the potato should be wary of having a negative effect on phenolic content. In one study, the concentration of anthocyanins in potatoes was adversely affected by gene manipulation designed to increase their methionine content (Dancs et al., 2008).

6.5.4 Analytical aspects

Total anthocyanin content can be measured colorimetrically by a differential method, taking advantage of the color changes induced by pH (Shahidi and Naczk, 2004). It is common to analyze the anthocyanidins (aglycones) by acid hydrolysis of the anthocyanins and subsequent chromatography (Merken et al., 2001). HPLC is used to identify the individual anthocyanins. Using analytical HPLC, Lewis et al. (1998) identified anthocyanins, flavonoids, and phenolic acids in the skin and flesh of the tubers, flowers, and the leaves of 26 cultivars of *Solanum tuberosum* L. This seminal study demonstrates a wide variation in anthocyanin levels of skin and flesh of different-colored tubers. The amounts in tubers reached up to 5 mg/kg fresh wt. The total glycoalkaloid content of red-fleshed potato breeding clones ranged (in mg/100 g tuber) from 2.0 to 36.3. Two cultivars with highest anthocyanin content contained the lowest levels of glycoalkaloids. To minimize anthocyanins degradation during the extraction, the authors recommend precipitating the glycoalkaloids in pH 8 solvent with an efficiency of 90% rather than subjecting the extracts to pH >9.2 for 100% efficiency (Rodriguez-Saona et al., 1998, 1999).

The purple potato, *Solanum tuberosum* cv. Congo, contained the anthocyanins petanin and the novel 3-O-[6-(4-ferulyl-O-α-rhamnopyranosyl)-β-glucopyranoside]-5-O-β-glucopyranosides of petunidin and malvidin (Fossen and Andersen, 2000). Solid phase extraction, countercurrent chromatography, preparative HPLC, HPLC-DAD, and LC-ESI-MS2 methods were successfully used to separate and characterize coumaric acid anthocyanin derivatives (3-ρ-coumaroylrutinoside-5-glucosides of petunidin, pelargonidin, peonidin and malvidin) from non-acylated anthocyanins as well as chlorogenic acids of pigmented potatoes (Eichhorn and Winterhalter, 2005). Analysis of 27 potato cultivars and four breeding clones showed that on average the highest amount (in g/kg fresh wt) of anthocyanins (0.65) were present in the skin followed by whole tubers (0.31) and flesh (0.22) (Jansen and Flamme, 2006). The 'Peru Purple' variety had the highest concentration in the skin (2.96).

Rates of nitrogen fertilization, year of harvest, and location of plant growth, and post-harvest storage for 135 days did not affect anthocyanin content of the tubers. The glycoalkaloid content

ranged as follows (in mg/100 g fresh wt): skin, 17.2; whole tubers, 4.4; and flesh, 2.3. Native South American potato cultivars contain high levels of anthocyanins. However, minerals in the soil appear not to significantly influence the phenolic content of purple potatoes (Andre et al., 2007).

6.5.5 Potential health benefits

Colored potatoes can significantly contribute to anthocyanin intake in the diet. Purple-fleshed potatoes contained 17–20 and red-fleshed 20–38 mg/100 g (Brown et al., 2005). This compares to 24–25 in red wine and 10–60 in the red raspberry (one of the highest in content). The authors suggest that potatoes could provide a cheaper source for anthocyanin extracts than other sources. Antioxidative activities of anthocyanin-containing potato extracts were higher than expected from anthocyanin content, suggesting synergistic effects among anthocyanins in the mixtures of the extracts (Giusti et al., 1999).

The antiviral effect of red-fleshed potato anthocyanins results from additive or synergistic effects of each anthocyanin pigment present in the mixture isolated from fresh potato hybrid (Hayashi et al., 2003). Anthocyanin-rich extracts and steamed red potatoes fed orally inhibited the growth of stomach cancer in mice (Hayashi et al., 2003, 2006). Unlike other reported studies on caspase-dependent anthocyanin-induced cell death (Hou et al., 2004), the cytotoxic activity against prostate cancer cells of an anthocyanin fraction from potatoes is due to activation caspase-independent apoptosis (Reddivari et al., 2007b). Consumption anthocyanin-rich red potato flakes had an antioxidant effect on serum lipid peroxidation and hepatic expression of superoxide dismutase mRNA in rats and protected against oxidative stress induced by a high cholesterol diet and liver injury (Han et al., 2006, 2007a, b).

6.6 Conclusions

The described methods for the analysis of biologically active anthocyanins, calystegine alkaloids, glycoalkaloids and hydrolysis products, and phenolic compounds in commercial potatoes, in processed potato products, and in new cultivars can lead to improvements in the precision and reliability of analyses for quality control and for safety of final products. Accurate analyses will benefit growers, researchers, processors, and consumers. Analytical studies have also facilitated concurrent studies of toxicities and of beneficial health effects as well as investigations of the biosynthesis of the secondary metabolites. Although potato processors are generally interested in imparting desirable sensory (organoleptic) attributes to potato products, the discovery of health-promoting effects of potato ingredients implies that analytical methodology will be paramount in future efforts designed to enhance the levels of these compounds in the human diet. Future studies should attempt to further simplify and improve the described analytical methodologies.

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